EVALUATION OF PERFORMANCE OF DOUGH AND BREAD INCORPORATING CHIA (*Salvia hispanica* L.)

Esther Iglesias-Puig and Monika Haros*

Cereal Group, Institute of Agrochemistry and Food Technology (IATA-CSIC),
Av. Agustín Escardino 7. Parque Científico, 46980 Paterna, Valencia, Spain

*Corresponding Author. Tel.: +34 96 390 00 22; Fax.: +34 96 363 63 01
e-mail: mharos@iata.csic.es (Monika Haros)

Abstract

As a result of the opinion given by the European Food Safety Authority about the safety of Chia seed (*Salvia hispanica* L) and whole ground Chia seed as food ingredients, they may be placed on the market in the European Community as novel food ingredients to be used in bread products. The objective of the present investigation was to develop new cereal-based products with increased nutritional quality by using chia and ground chia seeds (whole chia flour, semi-defatted chia flour and low-fat chia flour) in order to evaluate its potential as a bread-making ingredient. The samples with chia addition significantly increased the levels of proteins, lipids, ash and dietary fibre in the final product compared to the control sample. Breads with seeds or ground seeds showed similar technological quality to the control bread, except for the increase in specific bread volume, decrease in crumb firmness and change in crumb colour. Sensory analysis showed that the inclusion of chia increased overall acceptability by consumers. The thermal properties of the starch did not alter substantially with the inclusion of chia. However, the incorporation of chia inhibited the kinetics of amylopectin retrogradation during storage, which would be directly related to the delay in bread staling.

Keywords: chia, *Salvia hispanica* L., novel food, breadmaking ingredient, bakery products, bread performance

INTRODUCTION

Chia (*Salvia hispanica* L.) is a summer annual plant belonging to the *Labiatae* family [1]. Now that numerous studies have shown the remarkable nutritional properties of the chia seed, it is recommended for consumption because of its high oil content, protein, antioxidants, minerals and dietary fibre [2]. The seed has 25–38% oil, which has a high omega-3 and omega-6 content (mainly α-linolenic acid, 50–67%, and linoleic acid, 17–27%) in proper balance, of which it is a major plant source, making this ingredient a source of n-3 fatty acids [3,4]. In addition, chia contains a high proportion of natural antioxidant compounds (tocopherol, beta-carotene, chlorogenic acid, caffeic acid and flavonoids such as quercetin, myricetin, kaempferol), thus preventing rancidity of unsaturated fatty acids in food that contains it [5,6]. Chia seeds are also a source of riboflavin, niacin, thiamine and minerals such as calcium, phosphorous, potassium, zinc, magnesium and copper [7]. One of the most important aspects of this seed is its high fibre content; its use has important benefits such as the regulation of intestinal transit, reduction of the glycemic index and its corresponding insulin response, among others [6,8]. The fibre content of chia seed includes a polysaccharide gum with high molecular weight, mucilage. A structure of the basic unit of mucilage was proposed as a tetrasaccharide with 4-O-metil-α-D-glucoronopyranosyl residues occurring as branches of β-D-xylopyranosyl on the main chain [9]. Numerous epidemiological and experimental studies suggest that changes in the diet are important determinants in the prevention of various metabolic disorders included in the so-called metabolic syndrome, such as type 2 diabetes, insulin resistance, hypertension, obesity and cardiovascular disease. Furthermore, intake of food with high amounts of omega-3 leads to lower blood cholesterol and consequently lowers the risk of cardiovascular disease [10]. Therefore, chia seeds and their by-products could be ingredients of interest to enrich foods. As a result of the opinion given by the European Food Safety Authority about the safety of chia as a food ingredient [11], from 2009 chia seed and ground chia seed may be placed on the market in the European Community as a novel food ingredient to be used in bread products, with a maximum content of 5% chia seeds [12]. The designation of the novel food ingredient on the labelling of the foodstuff containing it is “Chia (*Salvia hispanica*) seeds” [11]. The daily bread intake recommended by the World Health Organization (250 g of bread/person) would result in an average intake of chia seeds of 12.5 g/person/day if all the bread consumed contained 5% chia seeds. However, the average consumption of bread in 17 member countries of the European Union is approximately 65 kg/person/year (or 178 g/person/day), with the highest consumption corresponding to Bulgaria, Czech Republic and Germany (110, 89 and 80 kg/year, respectively), whereas the lowest consumption is found in Finland (52 kg/year), United Kingdom and Norway (51 kg/year) and Sweden (50 kg/year), according to market reports of the Association Internationale de la Boulangerie Industrielle [13]. On the basis of this average bread consumption by adults in European countries, it is possible to estimate the average intake of chia seeds (9 g/person/day) if all the bread consumed contained 5% chia seeds. However, more recent studies indicate that bread consumption in Europe has decreased slightly to 170 g/person/day [13], which would reduce the estimate of average chia intake to 8.5 g/person/day.

Health and wellbeing are currently driving innovation in the bread sector. Bakers have responded to current trends in changing consumer tastes with the development of a wide choice of breads with added health benefits including prebiotics, n-3 fatty acids, and wholegrains, and high fibre and seeded breads. Consumer interest in health is reflected by sales figures for recent years, which show an increase in sales of white bread with added fibre, and of bread with added wholegrains. Continuing the health focus, salt reduction has also been a priority for the baking industry, because bread and cereal-based products contribute substantially to daily salt consumption [14]. The addition of dietary fibres - oligosaccharides, polysaccharides like gums, inulin and hemicellulose associated with lignin and other non-carbohydrate components- is one of the most important studies and market objectives [15] There are different strategies to increase the fibre content such as the addition of brans or flours like buckwheat, amaranth, rye, barley and soybean [16, 17, 18]. Another trend consists in the addition of antioxidants to bread as natural phenolic compounds which have different biological activities including anti-allergic, anti-viral, anti-inflammatory, anti-mutation and anti-cancer properties [19]. The replacement of wheat flour by legumes or pseudocereal flours give a prebiotic effect to bread products [20]. Seafoods are also being incorporated in bread to increase omega-3 fatty acid, chitin, chitosan, antioxidants, minerals, vitamins and bioactive compounds [20]. Encapsulation is an approach to incorporate omega-3 fatty acids in the dough [21]. It is possible to increase de polyunsaturated fatty acids content with the addition of flaxseed and quinoa flours [22]. Taking in to account that chia seed provides the majority of all these compounds with nutritional and functional properties - in terms of fibre, antioxidants, polyunsaturated fatty acids, minerals, vitamins and hydrocolloids, and being a vegetable source, it would be an excellent ingredient in bakery products.

Therefore the purpose of the present work was to provide further information on how replacing wheat flour by chia seeds and chia by-products at a 5% level affects mixing and overmixing properties, bread performance and overall acceptance by consumers, and to assess their functionality as ingredients with high nutritional value.

MATERIALS AND METHODS

Materials

Commercial Spanish wheat flour was purchased from the local market. The flour alveograph parameters were tenacity, P: 32 mm; extensibility, L: 130 mm; P/L ratio, 0.24; and deformation work, W: $139*10^{-4}$ J. Chia seeds WS, whole chia flour WF, semi-defatted chia flour SDF and low-fat chia flour LFF products were purchased from the ChiaSA Company (Valencia, Spain). The characteristics of the raw materials are shown in Table 1. Compressed yeast (*Saccharomyces cerevisiae*, Levamax, Spain) was used as a starter for the breadmaking process.

Determination of flour mixing behaviour

A Farinograph (Brabender, Duisburg, Germany) with a 300 g mixer was used to evaluate the impact of the chia ingredient in the flour on the mixing behaviour, following the official standard method with slight modifications [23]. The thermostat was maintained at 30 °C and all doughs were mixed in the Farinograph bowl to 500 Brabender Units (BU). The following parameters were determined in the Farinograph analysis: water absorption (percentage of water required to yield a dough consistency of 500 BU); arrival time (time for the curve to reach a consistency of 500 BU); dough development time (time to reach maximum consistency, min); stability (time during which dough consistency is kept at 500 BU, min); departure time (time for the curve to leave consistency of 500 BU); drop time (time elapsed from the beginning of mixing to a drop of 30 BU from the maximum consistency); and mixing tolerance index (consistency difference between height at peak and that at 5 min later, BU).

Breadmaking procedure

The control bread dough formula consisted of wheat flour (500 g), compressed yeast (2.5% flour basis), sodium salt (1.6% flour basis), tap water (up to optimum absorption, 500 Brabender Units) and ascorbic acid (0.01% flour basis). The ingredients were mixed for 4.0 min, rested for 10 min, divided (100 g), kneaded and then rested (15 min). Doughs were manually sheeted and rolled, proofed (up to optimum volume increase, at 28 °C, 85% relative humidity) and baked at between 190 °C/18 min and 170 °C/23 min, according to the formulation [24].

The chia ingredients were added at 5% on flour basis to the bread dough formula, providing the following samples: bread with 5% of chia seeds (WS), bread with 5% of whole chia flour (WF), bread with 5% of chia semi-defatted flour (SDF), bread with 5% of low-fat chia flour (LFF).

Fermentation was monitored by measuring pH, temperature and volume increase of the dough at regular intervals. After the fermentation step, the doughs were baked in an electric oven and cooled at room temperature for 75 min for subsequent analysis [24]. The experiments were done in duplicate.

Composition of raw materials and bread

Protein determination was carried out by the Kjeldahl technique [23]. Lipid content was extracted with petroleum ether under reflux conditions by the Soxhlet technique, whereas ash content was determined in a muffle by incineration at 910 °C. The dietary fibre content was measured by the total dietary fibre assay procedure [25].

Technological parameters

The technological parameters analysed were: loaf specific volume (cm^3/g), width/height ratio of the central slice or slice shape (cm/cm), moisture content (%) and the texture profile analysis using the TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, United Kingdom) [24]. Each parameter was measured at least in triplicate.

Digital image analysis was used to measure the bread crumb structure. Images were first scanned at 240 pixels per cm with a flatbed scanner (HP ScanJet 4400C, Hewlett Packard, USA) supported by HP PrecisionScan Pro 3.1 software. Two 10 mm x 10 mm square fields of view of the central slice (10 mm thick) of each of three loaves were used, thereby yielding 6 digital images for each baking. Data was processed using Sigma Scan Pro Image Analysis software (version 5.0.0, SPSS Inc., USA). The crumb grain features chosen were: cell area/total area, cm^2/cm^2; wall area/total area, cm^2/cm^2; number of cells per cm^2; and mean cell area, mm^2 [24].

The tristimulus colour parameters $L*$ (lightness), $a*$ (redness to greenness) and $b*$ (yellowness to blueness) of the baked loaves (crumb and crust) were determined using a digital colorimeter (Chroma Meter CR-400, Konika Minolta Sensing, Japan), previously calibrated with the white plate supplied by the manufacturer. The instrument settings were: illuminant C, display $L*$ $a*$ $b*$, and observer angle 10°. From the parameters determined, hue angle ($h*$), chroma ($C*$) and total colour difference ($\Delta E*$) were calculated by the equations: $h*_{ab} = \arctan(b*/a*)$; $C*_{ab} = (a*^2 + b*^2)^{1/2}$; $\Delta E* = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2}$. Each sample was measured 18 times at different points to minimize the heterogeneity produced by the chia ingredients.

Preliminary sensory analysis of the fresh breads was performed by a panel of 50 untrained tasters who usually consume wheat bread, using a hedonic scale of global acceptance (9. Like extremely; 8. Like very much; 7. Like moderately; 6. Like slightly;

5. Neither like nor dislike; 4. Dislike slightly; 3. Dislike moderately; 2. Dislike very much; 1. Dislike extremely).

Differential scanning calorimetry (DSC) analysis

The thermal properties of starch flour during baking of the fermented dough (gelatinization) and changes induced during the bread storage (amylopectin retrogradation) were measured on a calorimeter (DSC-7, Perkin-Elmer). Indium (enthalpy of fusion 28.41 J/g, melting point 156.4 °C) was used to calibrate the calorimeter. Fermented dough samples (30–40 mg) were weighed directly in DSC stainless steel pans (LVC 0319-0218, Perkin-Elmer) and hermetically sealed (Quick-Press, 0990-8467, Perkin-Elmer). The calorimeter scan conditions used were those of the methodology described by Leon et al. [26], later modified by Sanz-Penella et al. [24]. Briefly, to simulate the temperature profile in the centre of the bread crumb during baking, the samples were kept at 30 °C for 1 min, were heated from 30 to 110 °C at 11.7 °C/min, were kept at this temperature for 5 min, and cooled to 30 °C at 50 °C/min. To analyse amylopectin retrogradation, heated-cooled pans were stored at 20 °C for 0, 1, 2, 3, 5, 7 and 14 days, and heated again in the calorimeter from 30 to 130 °C, at 10 °C/min [27]. An empty pan was used as a reference and three replicates of each sample were analysed. Small endotherms of 1–2 J/g were observed at 90–112 °C. These thermal transitions were attributed to the amylose-lipid complex in cereal starches.

The parameters recorded were onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) of gelatinization and retrogradation transitions. Straight lines were drawn between T_o and T_c and the enthalpies associated with starch gelatinization and amylopectin retrogradation (ΔH_g and ΔH_r, respectively) were calculated as the area enclosed between the straight line and the endotherm curve. The enthalpies were expressed in Joules per grams of dough. The ratio $\Delta H_g/(T_p-T_o)$, designated as "peak height index" (PHI), was used to describe the relative shape of the endotherm [27].

Avrami model

Retrogradation enthalpy was fitted to the Avrami equation:

$$\theta = (A_\infty - A_t)/(A_\infty - A_o) = exp(-kt^n) \quad (1)$$

where θ is the fraction of the total change in the enthalpy of retrogradation still to occur. A_o, A_t and A_∞ are experimental values of the property at times zero, t and infinity (or limiting value), k is a rate constant, and n is the Avrami exponent. All the parameters were obtained from the modelling process.

Statistical analysis

Multiple sample comparison of the means and Fisher's least significant differences (LSD) were applied to establish significant statistical differences between treatments. All statistical analyses were carried out with the Statgraphics Plus 7.1 software (Bitstream, Cambridge, MN) and differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Raw material chemical composition

The chemical composition of the raw material used in this investigation is shown in Table 1. With regard to moisture content, chia seed and its products showed values between 3.85 and 6.41%. As was expected, the seed and its whole flour showed a high amount of lipids (32.48 to 33.90% in dry matter). According to the literature, chia seeds have a lipid content of about 25 to 39%, of which 60–70% is n-3 fatty acids [28]. The commercial samples of semi-defatted and low-fat chia flours clearly showed a significant decrease in fat content, which corresponded to a 56% and 80% reduction of lipids, respectively. This reduction promoted a significant increase in the protein fraction, ash and dietary fibre contents (Table 1). With the exception of moisture, the amounts of lipids, proteins, minerals and dietary fibre were significantly higher in chia than in wheat flour. Cereal flours contain high proportions of starch, while chia seed is virtually devoid of it. The presence of water-binding component or hydrophobic components in the dough formulation might alter the mixing and overmixing behaviour of hydrated flour, starch thermal properties and bread performance.

Effect of chia addition on dough mixing/overmixing properties

The Farinograph can be used to evaluate the flour-water absorption required to reach the defined dough consistency and to obtain the general profile of the dough during mixing and overmixing. During mixing, the distribution of materials, hydration and energy input for stretching and alignment of protein molecules involve shear and extensional deformation [24]. Flour-water absorption decreased significantly (p<0.05) with the inclusion of chia seeds, from 57.8 to 56.0% (Table 2). However, when chia flours were included the water absorption increased to values between 58.5 and 59.5%, despite the reduction of gluten content, in agreement with findings reported for fibres from various sources and hydrocolloids [16, 29]. This was mainly due to the high water-binding capacity of mucilage from fibre fraction, which is accessible in the WF, SDF and LFF samples. It is also important to note that the samples with semi-defatted and low-fat chia flours showed significantly higher water absorption than the WF sample (Table 2). In the particular case of seeds, which retained their integrity during mixing, release of mucilage to join the dough was inhibited, and therefore their ability to bind water was diminished. Furthermore, chia seeds had a higher particle size distribution than chia flours, which could be an additional factor to consider in the water absorption of the mixture, as was previously commented with regard to bran particle size [16]. During dough development a maximum dough consistency was reached and then the dough was able to resist deformation for some time, which determines the dough stability. The concomitant diluting effect of gluten proteins did not cause a decrease in either dough development time or mixing stability at 5% replacement of flour by chia ingredients, with the exception of WF and LFF samples (Table 2). The chia flours also produced a significant decrease in the drop time parameter, from 2.25 to 1.38 min, and a slight increase in the mixing tolerance index from 90 to 108 BU, which is not significant, revealing some resistance to overmixing such as the control sample.

Bread performance

The chemical composition of the breads supplemented with chia seeds and products thereof is presented in Table 3. The incorporation of chia in the formulation, whatever the ingredient, significantly increased protein, lipid and ash content and decreased the starch content with regard to the control sample. The greater levels of proteins, lipids and ash registered in the chia seeds and by-products with regard to the wheat flour directly affected the increase of these parameters in the bread, as expected. The same tendency was observed for loaf volume and total dietary fibre, modifying significantly from 282.5 to 361.7 mL and from 5.04 to 7.11%, respectively, with the replacement of wheat flour by chia. The higher specific volumes noticed in samples containing chia flours is owed to a better integration of mucilage to dough compared to the sample with chia seeds. Hydrocolloids – as mucilage – could improve loaf volume through the formation of hydrophilic complexes between their ionic groups and gluten proteins, and are also capable of establishing hydrophobic interactions. In general, these doughs were able to support a greater expansion during fermentation, reaching higher loaf volumes. In the case of seeds, which retained their integrity during the whole process, the interaction between mucilage and gluten network was inhibited, and therefore their ability to increase the specific volume. The moisture increase was fundamentally due to the inclusion of a greater amount of insoluble dietary fibre with chia. The mineral content increased significantly as a result of the replacement of wheat flour (ash: 1.99%) with chia by-products (ash: 2.40–2.51%), owing to the flour composition, as was expected.

Technological quality of fresh bread

The inclusion of chia flours produce breads with a significant increase in specific volume (Figure 1). The volume increase could be due to the interaction of the mucilage network, which interacts with the gluten network [30]. Hydrocolloids give a more porous structure to the gluten network, permitting greater stability and greater expansion of the dough during fermentation [30]. Nevertheless, the width/height ratio of the central slice or slice shape did not show significant differences in comparison with the control sample, although a decrease in this parameter was observed (Table 3). The texture parameters, crumb firmness, cohesiveness and chewiness, did not show significant differences. Nevertheless, it must be emphasized that firmness showed a tendency to decrease in products with chia ingredients. In general, the inclusion of hydrocolloids in bread doughs improves the texture profile of the crumb, reducing its hardness [30]. Nevertheless, in the current investigation the low amount of chia in bread formulation (5%) was insufficient for a positive effect on this parameter despite the increase of loaf volume. Chewiness represents the energy required to masticate a solid food product to a state ready for swallowing, and it is related to the primary parameters of firmness, cohesiveness, and elasticity [31]. In this investigation a decreasing trend in chewiness was observed with the inclusion of chia ingredients, as was also observed in crumb firmness (Table 3). On the other hand, cohesiveness did not show significant differences, which meant that chia ingredients did not affect the strength of the internal bonds making up the crumb, measured as the ratio of the peak area of the second compression divided by the peak area of the first compression [31]. Springiness was measured by the distance of the detected height of the product on the second compression divided by the original compression. Springiness, however, decreased slightly but significantly in the breads with a greater mucilage content, SDF and LFF, in comparison with the control sample, with values remaining close to 1. [32]. Resilience is calculated as the area during the withdrawal of the first compression divided by the area up to the maximum of the first cycle, which is related to the instantaneous ability of crumb for recovering the original geometry (instantaneous elasticity). This parameter in the loaves with seeds also showed significant differences with regard to the other samples, indicating a positive effect on the crumb matrix [32] (Table 3). This might be due to the high water retention capacity of the insoluble fibre –mainly mucilage- that chia contains [32].

The colour analysis of the crust of the products developed did not show significant differences with regard to lightness, hue and ΔE. However, chroma showed a slight and significant difference with respect to the control sample only in the sample with low-fat chia flour (Table 3). Greater differences were observed in the parameters that describe crumb colour. The bread products with chia flours showed lower values for lightness, chroma and hue, owing to the presence of the chia pigments. Chia contains phenolic compounds such as caffeic acid, chlorogenic acid, ferulic acid, p-coumaric acid, 7-hydroxycumarin, catechol, quercetin, quercetin-3-glucoside and kaempferol, which, in addition to their antioxidant activity, give the seeds their colour [6]. There were also significant differences in crumb colour, with values greater than 5 and therefore perceptible to the consumer. On the other hand, the inclusion of 5% chia by-products did not produce differences in the parameters that describe crumb structure (Table 3 and Figure 1).

Sensory evaluation

In this study, the overall acceptability of the products after tasting them was evaluated against a nine-point hedonic scale. Analysis indicated a high acceptance of the products by the tasters. The bread with chia seeds showed 97.8% of acceptance with a score of 7.75 on the hedonic scale, followed by the bread made with chia flours, with 90.2%–95.13% of acceptability and scores between 7.15 and 7.34; and, finally, the bread control obtained almost 70% acceptability and a score of 6.49 (Table 3). In general, the consumers indicated that the products had a low-salt taste. Salt reduction in manufactured foods is one of the Food Standards Agency's aims, and a challenge facing all EU governments and industries is to reduce salt intakes, the target salt level for bread being 1.1 g/100 g [14].

Effect of the inclusion of chia and chia by-products on thermal properties of starch in bread doughs

During the simulation of baking in the differential scanning calorimeter, we observed the peak corresponding to the process of partial gelatinization of the amorphous phase of starch, between 64.2 °C and 76.7 °C, with an enthalpy of 0.922 J/g of dough (Table 4). The range of gelatinization temperatures did not undergo significant changes with the incorporation of chia in the formulation, except in the peak temperature of the samples supplemented with defatted chia flour (SDF and LFF). The gelatinization enthalpy of the WS, WF and SDF samples did not show significant differences with respect to the control, but it was significantly different in the LFF sample. The presence of lipids generally interferes with the gelatinization of starch because of formation of the lipid-amylose complex, giving the starch greater granular stability, which is translated into greater gelatinization enthalpy [33]. The WF sample showed an increase in gelatinization enthalpy, which was significantly different from that of the defatted LFF sample. The integrity of the chia seeds would inhibit diffusion of the oil to the dough, and therefore these samples did not follow the same tendency. The samples supplemented with defatted flours showed values that were similar to the control or lower, because of the lower lipid concentration in them (Table 4).

In the second heating cycle, after storage at 20 °C, two peaks were observed. The first was the amylopectin retrogradation peak (peak temperature between 58.1 and 58.5 °C), while the second was the lipid-amylose complex fusion peak (peak temperature between 110.8 and 113.3 °C, data not shown). After 3 days of storage at 20 °C, the control sample retrogradation peak began at 46.5 °C and ended at 70.1 °C, with an enthalpy of 1.407 J/g of dough (Table 4). The chia ingredients produced practically no alteration in the transition temperature range, showing a tendency to reduce the final temperature. This reduction was significant in the sample with whole chia flour. Moreover, the retrogradation enthalpy tended to be lower in the samples with chia, significantly in the case of whole chia flour (Table 4). After the analysis of the thermal properties of the starch, the evolution of the amylopectin retrogradation was recorded, because it is one of the main mechanisms involved in bread staling (Figure 2). All the adjusted R^2 values for the curves of the retrogradation kinetics were in the range 0.991 to 0.999. This means that the equations can model the retrogradation enthalpy over time very precisely (Figure 2). The rate constant k in the equation describes the development of the retrogradation over time. A higher value of k means a faster retrogradation process. The Avrami exponent n indicates the nucleation and growth mode of the crystals [34]. The samples with seeds or with whole chia flour showed practically the same retrogradation kinetics as the control sample, but the limiting values according to the Avrami model were lower (1.812/1.829 J/g vs. 2.130 J/g). Ground chia is the ingredient with the highest accessible oil content and may interfere with the recrystallization of amylopectin (Table 4). This ingredient also has a high concentration of accessible mucilage, like the defatted flours, which affects the water balance in the dough and may inhibit retrogradation (Figure 2). In fact, the defatted samples (SDF and LFF) had lower retrogradation kinetics, with enthalpy limit values closer to, but lower than, the control sample (Table 4). Previous investigations concluded that the presence of hydrocolloids in bread doughs reduces retrogradation enthalpy. Hydrocolloids stabilize the water balance, and this, added to the interaction with the gluten network, makes the recrystallization of amylopectin more difficult [32, 35].

Conclusions

The incorporation of 5% chia could increase the nutritional value of bread products with regard to the concentrations of proteins of greatest biological value, lipids with a high proportion of omega fatty acids and dietary fibre. The chia ingredients produced practically no alteration in the mixing and overmixing properties, with the exception of water absorption, mainly because of the presence of mucilage. There were no differences in the quality of all the products developed with chia in comparison with the control sample, except for the increase in loaf specific volume and the change in crumb colour. However, chia seeds and chia by-products increased the general acceptability of the bread products, despite their reduced salt content. Throughout the process, the thermal properties of the starch did not alter substantially with the inclusion of chia. However, the incorporation of chia inhibited the kinetics of amylopectin retrogradation during storage, which would be directly related to the delay in bread staling. The inclusion of chia seeds or flours had a positive effect on the technological and sensory value of the bread products, and therefore its inclusion is recommended, even at levels greater than 5%.

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FIGURE CAPTIONS

Fig. 1 Effect of the inclusion of chia by-products on loaf shape, central slice and crumb structure. Bread formulations: **a**, control bread; **b**, bread with 5% of chia seeds; **c**, bread with 5% of whole chia flour; **d**, bread with 5% of chia semi-defatted flour; **e**, bread with 5% of low-fat chia flour.

Fig. 2 Effect of chia on kinetics of amylopectin retrogradation. Bread formulations: *black circles*, control sample; *triangles*, bread with 5% of chia seeds; *squares*, bread with 5% of whole chia flour; *diamonds*, bread with 5% of chia semi-defatted flour; *white circles*, bread with 5% of low-fat chia flour. Lines correspond to Avrami model.

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Table 1. Chemical composition of raw materials used in this study

Material	g/100 g in dry matter				
	Moisture	**Lipids**	**Proteins**	**Ash**	**Total Dietary Fibre**
Wheat Flour	14.27±0.01	0.8±0.3	10.1±0.1	0.64±0.01	5.3±0.2
Chia Seeds	3.85±0.01	33.9±0.2	20.2±0.4	2.3±0.4	30.9±0.5
Whole Chia Flour	6.41±0.01	32.5±0.5	20.0±0.4	2.2±0.5	36.2±0.3
Semi-Defatted Chia Flour	4.33±0.01	18.6±0.7	22.5±0.9	3.53±0.04	34.3±0.1
Low-Fat Chia Flour	4.80±0.01	6.8±0.4	23.5±0.1	4.90±0.09	38.4±0.1

[a-e]Mean, n=3. Values followed by the same letter in the same column are not significantly different at 95% confidence level

Moisture on wet basis

Table 2. Effect of chia by-product addition on dough mixing/overmixing properties

Sample	**Units**	**Control**	**Chia**			
			Seeds	**Whole Flour**	**Semi-Defatted**	**Low-Fat**
Water Absorption	%	57.8 ^{b}	56.0 ^{a}	58.5 ^{b}	59.5 ^{c}	59.5 ^{c}
Arrival Time	min	1.25 ^{a}	1.00 ^{a}	1.38 ^{a}	1.00 ^{a}	1.00 ^{a}
Dough Development Time	min	2.75 ^{ab}	2.63 ^{ab}	3.75 ^{b}	2.50 ^{a}	3.00 ^{ab}
Stability	min	3.50 ^{a}	3.75 ^{ab}	4.00 ^{b}	3.63 ^{ab}	4.25 ^{b}
Departure Time	min	4.75 ^{a}	4.75 ^{a}	5.38 ^{a}	4.63 ^{a}	4.58 ^{a}
Mixing Tolerance Index	BU	90 ^{a}	95 ^{a}	108 ^{a}	105 ^{a}	100 ^{a}
Drop Time	min	2.25 ^{b}	1.50 ^{a}	1.38 ^{a}	1.50 ^{a}	1.75 ^{ab}

$^{a-c}$Mean, n=3. Values followed by the same letter in the same line are not significantly different at 95% confidence level

BU, Brabender Units

Table 3. Effect of chia by-product addition on bread performance

Sample	Units	Control	Chia			
			Seeds	Whole Flour	Semi-Defatted	Low-Fat
Physico-Chemical Parameters						
Moisture	%	34.80 ^{a}	33.73 ^{a}	33.92 ^{a}	35.98 ^{a}	35.69 ^{a}
Loaf Volume	mL	282.5 ^{a}	285.0 ^{a}	324.2 ^{b}	330.8 ^{b}	361.7 ^{b}
Specific Volume	mL/g	3.29 ^{a}	3.58 ^{a}	4.08 ^{b}	4.12 ^{b}	4.53 ^{b}
Shape Ratio	cm/cm	1.79 ^{a}	1.71 ^{a}	1.61 ^{a}	1.62 ^{a}	1.73 ^{a}
Proteins	% d.m.	16.1 ^{a}	16.9 ^{b}	17.1 ^{b}	16.9 ^{b}	17.2 ^{b}
Total Dietary Fibre	% d.m.	5.04 ^{a}	6.82 ^{b}	6.27 ^{b}	6.98 ^{b}	7.11 ^{b}
Lipids	% d.m.	0.25 ^{a}	2.11 ^{c}	2.21 ^{c}	0.91 ^{b}	0.57 ^{ab}
Ash	% d.m.	1.99 ^{a}	2.47 ^{b}	2.40 ^{b}	2.51 ^{b}	2.42 ^{b}
Crumb Textural Parameters						
Firmness	N	1.530 ^{a}	1.457 ^{a}	1.190 ^{a}	1.168 ^{a}	1.310 ^{a}
Springiness		1.000 ^{b}	0.990 ^{ab}	0.985 ^{ab}	0.973 ^{a}	0.973 ^{a}
Cohesiveness		0.882 ^{a}	0.882 ^{a}	0.899 ^{a}	0.865 ^{a}	0.893 ^{a}
Chewiness	N	1.326 ^{a}	1.264 ^{a}	1.030 ^{a}	1.012 ^{a}	1.222 ^{a}
Resilience		0.501 ^{a}	0.543 ^{b}	0.517 ^{a}	0.515 ^{a}	0.514 ^{a}
Crust Colour Parameters						
L^*		67.5 ^{a}	68.9 ^{a}	68.3 ^{a}	68.2 ^{a}	67.8 ^{a}
C^*		33.8 ^{a}	32.9 ^{ab}	30.9 ^{a}	31.7 ^{ab}	30.7 ^{b}
h_{ab}		82.7 ^{a}	84.3 ^{a}	80.9 ^{a}	83.7 ^{a}	83.9 ^{a}
ΔE		---	4,14 ^{a}	4,55 ^{a}	4,39 ^{a}	5,12 ^{a}
Crumb Colour Parameters						
L^*		66.2 ^{d}	63.0 ^{c}	60.5 ^{ab}	59.0 ^{a}	61.5 ^{bc}
C^*		14.0 ^{c}	12.3 ^{a}	12.5 ^{ab}	11.9 ^{a}	13.1 ^{b}
h_{ab}		96.6 ^{c}	95.3 ^{b}	91.1 ^{a}	91.6 ^{a}	91.1 ^{a}
ΔE		---	4,20 ^{a}	6,43 ^{bc}	7,69 ^{c}	5,20 ^{ab}
Crumb Grain (Digital Image Analysis)						
Cell Area/Total Area	cm^2/cm^2	0.198 ^{a}	0.151 ^{a}	0.205 ^{a}	0.158 ^{a}	0.172 ^{a}
Wall Area/Total Area	cm^2/cm^2	0.802 ^{a}	0.849 ^{a}	0.795 ^{a}	0.842 ^{a}	0.828 ^{a}
Cells/cm^2		19.1 ^{a}	22.8 ^{a}	23.3 ^{a}	22.8 ^{a}	22.5 ^{a}
Mean Cell Area	mm^2	1.009 ^{b}	0.825 ^{ab}	0.877 ^{ab}	0.721 ^{a}	0.788 ^{ab}
Maximum Cell	mm^2	7.12	6.08	7.61	5.87	6.13
Sensory Evaluation (Hedonic Scale)						
Overall Acceptability		6.49 ^{a}	7.75 ^{c}	7.15 ^{b}	7.34 ^{bc}	7.25 ^{b}

$^{a\text{-}d}$Means within lines followed by the same letter are not significantly different at 95% confidence level

d.m., dry matter

Table 4. Effect of chia by-product addition on starch thermal properties

Sample	Units	Control	Chia			
			Seeds	**Whole Flour**	**Semi-Defatted**	**Low-Fat**
Starch Gelatinization						
Onset Temperature	°C	64.2 [a]	64.2 [a]	64.0 [a]	64.5 [a]	64.5 [a]
Peak Temperature	°C	69.8 [ab]	69.9 [ab]	69.6 [a]	70.1 [b]	70.1 [b]
Conclusion Temperature	°C	76.7 [a]	76.8 [a]	76.6 [a]	77.3 [b]	76.7 [a]
Gelatinization Enthalpy	J/g	0.922 [bc]	0.863 [ab]	0.975 [c]	0.921 [bc]	0.818 [a]
PHI	J/g °C	0.168 [c]	0.151 [ab]	0.174 [c]	0.165 [bc]	0.146 [a]
Amylopectin Retrogradation, 3 days						
Onset Temperature	°C	46.5 [a]	46.7 [a]	46.7 [a]	46.8 [a]	46.5 [a]
Peak Temperature	°C	58.5 [a]	58.5 [a]	58.1 [a]	58.3 [a]	58.3 [a]
Conclusion Temperature	°C	70.1 [b]	69.7 [ab]	69.2 [a]	69.6 [ab]	69.9 [b]
Retrogradation Enthalpy	J/g	1.407 [b]	1.249 [ab]	1.079 [a]	1.250 [ab]	1.294 [ab]
Avrami parameters						
$\Delta H_{r\infty}$	J/g	2.130	1.812	1.829	1.950	2.025
n		0.943	1.089	0.828	0.990	1.034
k		0.380	0.379	0.386	0.352	0.274
R^2		0.999	0.991	0.991	0.991	0.994

[a-c]Means within lines followed by the same letter are not significantly different at 95% confidence level

DSC, differential scanning calorimeter; PHI, peak height index $\Delta H_g/(T_p-T_o)$; ΔH_{ro}, retrogradation enthalpy at zero time; ΔH_{rt}, retrogradation enthalpy at "*t*" time; $\Delta H_{r\infty}$, retrogradation enthalpy at ∞ time; *k*, rate constant; *n*, Avrami exponent; R^2, adjusted regression coefficient; Avrami equation

$$\frac{\Delta H_{r\infty} - \Delta H_{rt}}{\Delta H_{r\infty} - \Delta H_{ro}} = e^{-kt^n}$$